植物病原細菌分類與鑑定技術-以軟腐細菌為例

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摘要

近年隨著植物病原細菌研究的進步，在分類上出現明顯的變化，以原屬於 *Erwinia* 之軟腐細菌為例，已被拆分至 *Pectobacterium* 及 *Dickeya* 屬，其內一些亞種被提升至種的層級，新種陸續不斷的發表。為滿足日益複雜的病原細菌鑑定需求，除了傳統鑑定技術外，利用次世代定序、基因組裝、數位化核酸雜合(digital DNA-DNA hybridization, dDDH) 及平均核苷酸相似度計算 (average nucleotide identity, ANI)，可獲得具信賴的數據，應用在鑑定屬內相近的細菌種類，已廣泛為學者採用。

關鍵詞：分類、鑑定、軟腐細菌、數位化核酸雜合、平均核苷酸相似度計算

前言

近年由於植物病原細菌研究的發展，植物病原細菌分類不斷的改變，從 Laboratory guide for identification of plant pathogenic bacteria 一書中，可知重要植物病原細菌，可分為革蘭氏陽性菌與革蘭氏陰性菌，在革蘭氏陰性菌中，主要的病原細菌有 *Erwinia*、*Pantoea*、*Pseudomonas*、*Acidovorax*、*Ralstonia*、*Burkholderia*、*Agrobacterium*、*Xanthomonas*、*Xylella* 等屬 (Schaad *et al.* 2001)。革蘭氏陽性菌主要有 *Clavibacter*、*Streptomyces* 等屬。然而近年來由於分子生物學以及生物資訊學的進步，細菌分類地位不斷的更動。在屬 (genus) 的層級上，*Erwinia* 陸續被拆分成了 5 個屬包括 *Erwinia*、*Pectobacterium*、*Dickeya*、*Pantoea* 及 *Brenneria*。此外在種 (species) 的層級上則不斷的有新物種發表以及種名改變。在細菌學名分類快速變動的情況下，各國在農產品進出口檢疫勢必因病原細菌學名的變化而產生檢疫條件及措施的改變，因此在國際貿易檢疫的工作上，需不斷的蒐集國際的疫情資料，即時的更新病原細菌的學名，在

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國內防疫工作，亦須掌握國內已存在的植物病原菌種類，以因應日益複雜的農產品進出口檢疫及國內病害防疫工作。

一、軟腐細菌分類地位的變化

細菌分類學和命名法可參考原核生物系統學國際委員會(International Committee on Systematics of Prokaryotes, ICSP)所訂定之國際原核生物命名法規(International Code of Nomenclature of Prokaryotes, ICNP)。細菌學名需發表在國際分類期刊International Journal of Systematic and Evolutionary Microbiology (IJSEM) 才被視為有效發表。發表在 IJSEM 以外的學術期刊細菌學名則需要再經過 IJSEM 審核確認並公告於 IJSEM 的 Validation Lists 清單之後，才能成為有效學名。

在重要植物病原細菌中，植物軟腐細菌原屬於 *Erwinia* 屬，Hauben 等人 (1998) 依照 16S rDNA 序列的差異，將其歸入 *Pectobacterium carotovorum* subsp. *carotovorum*、*P.c.* subsp. *atrosepticum*、*P. c.* subsp. *betavasculorum*、*P. c.* subsp. *odoriferum*、*P. c.* subsp. *wasabiae* 5 個亞種以及 *P. cacticidum*、*P. chrysanthemi* 、*P. cypripedii* 3 個種。Gardan 等人 (2003) 進一步依據 16S rDNA 序列、血清學及DNA-DNA 雜合反應等結果，再將 *P. carotovorum* 中 3 個亞種提升至種的地位，分別為 *P. atrosepticum*、*P. betavasculorum* 與 *P. wasabiae*。之後仍不斷有 *P. c.* 的新亞種被報導，包括有 *P. c.* subsp. *brasiliensis* (Duarte *et al.* 2004; El Tassa and Duarte 2004) 和 *P. c.* subsp. *actinidiae* (Koh *et al.* 2012)。Zhang 等人 (2016) 以 *is*DDH 以及 ANI 數值分析 *Pectobacterium* 屬細菌，認為應將 *Pectobacterium carotovorum* (*P. c.*)之 4 個亞種 (subspecies)，包括 *P. c.* subsp. *actinidiae*, *P. c.* subsp. *odoriferum*, *P. c.* subsp. *carotovorum* 及 *P. c.* subsp. *brasiliense* 提升至種的層級，並發現有成為新種的潛力菌株。在 2019 年，*P. odoriferum*、*P. brasiliense*、*P. actinidiae*、*P. versatile* 在 IJSEM 期刊發表，並修正 *P. carotovorum* 的描述 (Portier *et al.* 2019)。至 2021 年 *Pectobacterium* 已達到了 20 個種 (Zhou *et al.* 2022)。

另一重要的 *Dickeya* 屬之植物軟腐細菌，原為 *Pectobacterium chrysanthemi* ，Samson 等人 (2005) 以 16S rDNA 序列、表現型數值、血清學特性及 DNA-DNA 雜合反應等資料，成立 *Dickeya* 屬並將 *P. chrysanthemi* 重新分配到 6 個新種，分別為 *D. chrysanthemi*、*D. dadantii*、*D. dianthicola*、*D. dieffenbachiae*、*D. paradisiaca*、*D. zeae*。其中 *D. dieffenbachiae* 後續再被重新分類為 *D. dadantii* subsp. *dieffenbachiae* (Brady *et al.* 2012)。之後不斷有學者發表 *Dickeya* 屬之新物種，包括有 *D. aquatic* (Parkinson *et*

al. 2014)、*D. solani* (van der Wolf *et al.* 2014)、*D. fangzhongdai* (Tian *et al.* 2016)、*D. lacustris* (Hugouvieux-Cotte-Pattat *et al.* 2019)、*D. undicola* (Oulghazi *et al.* 2019)、*D. poaceiphila* (Hugouvieux-Cotte-Pattat *et al.* 2020)、*D. oryzae* (Wang *et al.* 2020)、*Dickeya parazeae* (Hugouvieux-Cotte-Pattat and Van Gijsegem 2021) 等，至 2021 年 *Dickeya* 屬之物種已達到 13 種。

在台灣重要且寄主範圍廣泛的軟腐細菌，主要有 *P. c.* subsp. *carotovorum* (Pcc) 及 *P. chrysanthemi* (Pch) (Huang *et al.* 2007)，隨著學名不斷的變動，經學者研究台灣在 *Pectobacterium* 屬存在之物種有 *P. brasiliense*、*P. versatile*、*P. aroidearum* 等 3 種以上 (Chen *et al.* 2020; Kao *et al.* 2022)。*Dckeya* 屬有 *D. dadantii*, *D. chrysanthemi*, *D. undicola*, and *D. fangzhongdai* (Tsai *et al.* 2019; Wei *et al.* 2021)。

二、植物病原細菌的鑑定技術

植物病原細菌鑑定方式，早期傳統上以細菌形態、菌落培養特徵、生理生化特性之表現型特徵 (phenotypic characteristics) 分析，隨著生化及分子生物技術的進步，API 細菌鑑定套組、Biolog 細菌鑑定系統、細菌脂肪酸分析、基質輔助雷射解吸電離飛行時間質譜 (matrix-assisted laser desorption/ionization time of flight mass spectrometry, MALDI-TOF MS)之蛋白質圖譜分析並輔以 DNA/DNA 雜合技術、細菌特定基因序列分析如 16S rDNA、ITS DNA 及管家基因 (housekeeping gene) 等之基因型特徵 (genotypic characteristics)，有助於釐清以傳統表現型特徵分析上，不易區分的之細菌種類，此種以多相數據進行互相印證方式，即為多相分類 (polyphasic taxonomy)。

在細菌分類鑑定，同一屬內之近似種或同一種內之亞種細菌的鑑定，有時不易以傳統的表現型特徵進行確認，大多須再採用核酸序列分析輔以證明，目前學者廣泛採用的方式有細菌特定基因序列的比對以及利用多基因序列分析 (multilocus sequence analysis, MLSA)，以多個具有保守功能的蛋白質部分基因序列之管家基因，建立細菌的親緣關係圖，用以分辨近似的細菌物種 (Glaeser and Kämpfer 2015)。

近來由於次世代定序 (next-generation sequencing, NGS) 技術的發展，該技術所產生的細菌 DNA 短片段可經生物資訊軟體進行 de novo 組裝成基因體草圖 (draft genome sequence)，得到近乎細菌全基因體序列，此細菌基因體序列可經數位化核酸雜合 (dDDH) 或平均核苷酸相似度計算 (ANI)，可進一步對物種進行分辨。dDDH 的臨界值 (threshold) 為 70%，ANI 的臨界值 95-96%。(Lee *et al.* 2016; Meier-

Kolthoff *et al.* 2013)。目前 NGS 技術發展越趨成熟，成本已逐漸降低至一般實驗室可負擔的水平，利用該技術可得到具信賴的數據，因此逐漸廣為各領域學者應用，近年新發表的細菌植物病原細菌新種，或原為亞種的細菌提升至種的層級，大多須具備 MLSA、dDDH 及 ANI 的數據分析，如上節所提到革蘭性陰性菌之軟腐細菌 *Pectobacterium* 及 *Dickeya* 屬的多種新種細菌的發表。另外革蘭性陽性菌中之重要國際檢疫細菌 *Clavibacter michiganensis* (*C. m.*)的多個亞種包括 *C. m.* subsp. *sepedonicus*、*C. m.* subsp. *insidiosus*、*C. m.* subsp. *nebraskensis*、*C. m.* subsp. *tessellarius*、*C. m.* subsp. *capsici*，以更多數據佐證後，提升至種的層級 *C. sepedonicus*、*C. insidiosus*、*C. nebraskensis*、*C. tessellarius*、*C. capsici* (Li *et al.* 2018)，且仍不斷有新種發表 *Clavibacter zhangzhiyongii* (Tian *et al.* 2021)。

總結

隨著次世代定序技術與生物資訊技術的進步，顯示以生物資訊學(bioinformatics)分析細菌全基因體已成為細菌鑑定上的重要方式。被發表的病原細菌物種不斷增加且學名的變動，造成細菌的分類漸趨複雜，國際農產品檢疫勢必採用有效發表的學名作為依據，因此蒐集最新的國際疫情、增加國際學術合作、生物資源的交流、掌握國內病原細菌種類，並建立重要檢疫細菌鑑定技術，方能與國際接軌，以處理我國農產品進出口的檢疫問題。此外利用次世代定序所得的龐大數據中，可經由基因註釋，從中發掘有用的基因組序列資訊，可用來開發病原細菌快速偵測技術以供國內防疫與邊境檢疫的時效性需求。

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Classification and identification technology of plant pathogenic bacteria-A case of soft rot bacteria

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Abstract

In recent years, with the development and progress of research on plant pathogens, their classification has undergone significant changes. For example, the soft-rot bacteria that originally belonged to *Erwinia* were divided into the genera *Pectobacterium* and *Dickeya*. Some of the subspecies were elevated to species and new species were published constantly. In order to meet the increasingly complex identification needs of pathogenic bacteria, in addition to traditional identification techniques, next generation sequencing, gene assembly, digital DNA-DNA hybridization (dDDH) and average nucleotide identity (ANI) can obtain reliable data to identify similar species within the same genus that has been widely used by scholars.

Key words: classification, identification, soft rot bacteria, dDDH, ANI

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